SUB D13 CONT. C8

83. (New) A method for treating cancer which is sensitive to immunotherapy comprising:

(a) removing diseased cells from a mammal;

(b) increasing or decreasing the expression of antigen by the cell; and immunizing the mammal with an effective amount of the cell to prevent or alleviate the symptoms of the disease wherein the treatment involves activation or maturation of dendritic cells or peripheral blood macrophages pulsed with antigen in the form of protein, peptide, mRNA encoding antigen, or DNA encoding antigen from tumor cells.

Remarks

A. General Comments

The instantly claimed invention teaches methods to elicit antigen presentation by cells which normally do not carry out that function and to enhance and improve antigen presentation in cells that do carry out that function such as dendritic cells. It teaches generally how to cause an increase in genes important in antigen presentation by artificially introducing sequence non-specific double stranded polynucleotides to cells. Among the objects of the invention is to increase the expression of immune response recognition molecules, and to exploit the expression for the treatment of specific diseases.

The instantly claimed invention also teaches that sequence non-specific double-stranded polynucleotides greater than 25 nucleotides in length activate the expression of immune recognition molecules in cells. It teaches a simple and specific system to activate expression of Class I and/or Class II molecules of the major histocompatibility complex (MHC), and allows regulation of expression of MHC molecules on the cell-surface of antigen presenting cells and other immune cells.

B. Nonstatutory Subject Matter Rejection

The Examiner rejected Claims 1, 2, 4, 6, 7, 9-13, 15, 17, 18, 23-26, 42, 43 and 44 under 35 U.S.C. § 101 as being directed to nonstatutory subject matter. The Examiner stated that the "the claims are drawn to a method of increasing immune recognition of a mammalian cell by

introducing a sequence non-specific double stranded polynucleotide greater than 25 nucleotides in length into the cell and thereby activating expression of a gene or gene product that increases immune recognition gene or gene product, peptide processing gene or gene products, Class II regulatory genes and gene products, costimulatory molecule gene or gene products, wherein such activation is involved in antigen presentation, growth and function of the cell and which increases the ability of a cell to present antigen to an immune cell." The Examiner further stated that the "claimed method is indistinguishable from naturally occurring viral or bacterial infection processes and a naturally occurring injury process causing the leakage of self DNA fragments into the cell cytoplasm."

Applicants have amended independent Claim 1 so as to not embrace naturally occurring processes. Applicants respectfully submit that in view of this amendment Claim 1 as amended and dependant Claims 2, 4, 6, 7, 9-13, 15, 17, 18, 23-26, 42, 43 and 44 are presently in condition for allowance.

C. Enablement Rejection

The Examiner rejected Claims 1, 2, 4-18, 21-26, 29-35, 42-46, 74 and 75 under 35 U.S.C. § 112, first paragraph, stating that the specification:

> "does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims."

Applicants respectfully disagree with the Examiner's statement that the claims are not enabled. Applicants' invention teaches a detailed description of how to create an autoimmune disease mimicking Graves disease in a mouse model. This detailed disclosure is discussed on page 94 of the specification in the 3 references by the Shimojo group. (Shimojo, N., Kohno, Y., Yamaguchi, K-I., Kikuoka, S-I., Hoshioka, A., Niimi, H., Hirai, A,, Tamura, Y., Saito, Y.,

Kohn, L. D., and Tahara, K. (1996). Proc. Natl. Acad. Sci. U.S.A. 93:11074-11079; Yamaguchi, K-I., Shimojo, N., Kikuoka, S., Hoshioka, A., Hirai, A., Tahara, K., Kohn, L. D., Kohno, Y., and Niimi, H. (1997) J. Clin. Endocrinol. Metab. 82:4266-4269; and Kikuoka, S., Shimojo, N., Yamaguchi, K-I., Watanabe, Y., Hoshioka, A., Hirai, A., Saito, Y., Tahara, K., Kohn, L. D., Kohno, Y., and Niimi, H. (1998) Endocrinology 139:1891-1898; copies enclosed herewith). The Shimojo model procedure has also been repeated by the Davies group (Kita, M., Ahmad, L., Marians, R. C., Vlase, H., Unger, P., Graves, P. N., and Davies, T. F. (1999) Endocrinology 140:1392-1398; copy enclosed herwith). Applicants submit that this is the same procedure used to create the Graves model disclosed in the instant patent application on page 94. Applicants teach that this procedure develops an *in vivo* protective immune response in a mouse system and therefore can be adapted to develop protective immune responses which require a comparable in vivo immune response in other animal models including humans.

The Examiner has also incorrectly asserted that there is not enough guidance to induce *any and all* autoimmune reactions. Applicants respectfully disagree with the Examiner since the instant invention provides sufficient disclosure that the instantly claimed invention produces a protective response in tumors (the thyroid tumor model) and can generate *in vivo* antibody immune responses. Applicants have reported this in the reference by Iishi and co-workers (copy attached herewith).

The specification provides dosage amounts, frequency of immunization (6 times), route of administration (intraperitoneal or intramuscular) which are described in detail in the Shimojo references (copies attached hereto) and cited on page 94 of the patent application.

The Examiner's further asserts that there is not enough of a disclosure to correlate with protective or therapeutic immune responses. Applicants respectfully disagree with the Examiner

as it is contrary to the teachings of the instant invention. Applicants have practiced the instantly claimed invention and used the identical procedure to express tumor autoantigens that prevent a thyroid tumor from developing or growing as well as a standard injection procedure to develop an antibody response that exceeds a known adjuvant protocol. This research is disclosed in the references attached hereto. This guidance therefore existed and did not require undue experimentation as evidenced by Applicants own publications. Applicants respectfully submit that because some experimentation may be required, that level of experimentation does not rise to the level of undue experimentation.

The Examiner's assertion that the instant invention is not applicable to a protective or therapeutic response suggests an incomplete understanding of the model of autoimmunity relative to a protective or therapeutic response. Applicants respectfully submit that autoimmunity is a protective response. The response to infection, injury, and related insults that cause double-stranded polynucleotides to be introduced into the cytoplasm can be is a normal host defense mechanism that protects the organism from infection or cell injury. It is clear that the double-stranded DNA is communicating to the cell that there is something wrong *e.g.*, that the cell is infected with a virus, the cell is injured, or the cell has an oncogenic transformation which causes leaking of nuclear DNA. Thus, the cell is searching for a protective response by the organism.

The required protective response is to allow immune cells or autoantibodies to form which destroy the cell and arrest the damage or disease process. Pathologic autoimmunity develops if the insult is overwhelming or if there is another lesion which is often genetic. In some instances, overwhelming infections or specific viruses, for example, single strand RNA viruses, which replicate in the cell and use Y-box proteins or SSBP-1 in that process may

contribute to the development of autoimmune disease. In the case of viruses, the virus captures host transcription factors for their replicative process. The association of thyroid autoimmunity with hepatitis, foamy virus, and related negative single stranded RNA viruses is consistent with that research data. Genetics will contribute to disease susceptibility subsequent to the initial inciting event and bystander immune cell reaction.

Genomic screening has shown that multiple loci can contribute to disease expression and that these loci are not unique to thyroid autoimmunity. One such loci is the cytotoxic T lymphocyte-associated 4 (*CTLA-4*) gene. This research is discussed in the Heward reference. (Heward, J. M., Allahabadia, A., Armitage, M., Hattersley, A., Dodson, P. M., Macleod, K., Carr-Smith, J., Daykin, J., Daly, A., Sheppard, M. C., Holder, R. L., Barnett, A. H., Franklyn, J. A., and Gough, C. L. (1999) J. Clin. Endocrinol. Metab. 84:2398-2401; copy enclosed herewith). This gene is generally important in regulating self-tolerance by the immune system and in the pathogenesis of multiple autoimmune disorders, not only Graves disease. An individual with such a defect, who is exposed to an infection or an environmental tissue injury affecting the thyrocyte, could, therefore, convert a host protective response to an autoimmune disease. Recent research mapping of the major susceptibility loci for familial Graves disease and Hashimoto's disease suggests there is genetic heterogeneity and a complex interaction between predisposing genes and environmental triggers. This research is disclosed in the reference by the Tomer group. (Tomer, Y., Barbesino, G., Greenberg, D. A., Concepcion, E., and Davies, T. F. (1999) J. Clin. Endocrinol. Metab. 84:4656-4664; copy enclosed herewith).

Additionally, the significance of associations of MHC class I haplotypes, i.e. B35, with Graves disease is clear proof of a genetic predisposition for this diseaese. Of interest, with respect to the hypothesis that this is a protective mechanism is the association of B35 and an

infection by foamy viruses with DeQuervain's disease, an immune form of hyperthyroidism-thyroiditis. This research is also discussed in a reference by the Tomer group. (Tomer, Y., and Davies, T. (1993) Endocr. Rev. 14:107-121; copy enclosed herewith).

Thus, contrary to the Examiner's assertion it is reasonable to assume that an autoimmune response is a protective response. This assumption fits with evidence that immune cells in individuals with an autoimmune disease contain the same repertoire as in normal individuals. Multiple groups, for example, had used TSHR peptides to develop a profile of T cells reactive with APCs in Graves disease patients. The Moltini group (which included Dr. Leonard D. Kohn, one of the Applicants as a co-author), demonstrated that T cells from normal individuals exhibited effectively the same TSHR peptide profile as did Graves disease patients. (Molteni, M., Zulian, C., Scrofano, S., Della Bella, S., Bonara, P., Kohn, L. D., and Scorza, R. (1998) Thyroid 8:241-247; copy enclosed herewith). This was consistent with the model which suggested that T cells from normal individuals had the full potential to react to TSHR if presented in the context of an APC.

One of the inventor's, Dr. Leonard D. Kohn co-authored a manuscript which disclosed T cells from normal individuals that were reactive with some of the TSHR peptides. (Molteni, M., Kohn, L. D., Scrofani, S., Bonara, P., and Scorza, R. (2000) Induction of anergy in TSHR-specific clones by CD8+ cells; Manuscript submitted; copy enclosed herewith). Three types of cells were cloned: a CD4+ cell which reacted to TSHR-pulsed APCs with a greater than 10-fold increase in thymidine incorporation; a CD4+ cell which reacted to the TSHR-pulsed APCs with a 2-3-fold increase in thymidine incorporation; and a CD8+ cell which did not respond to the TSHR-APCs with an increase in thymidine incorporation. Several points were noted in this

manuscript when the different cells in these studies of normal individuals were further characterized.

First, the reactivity of low reactive CD4+ cells to TSHR-APCs decreased with time in culture; however, their activity could be restored by freeze-thaw procedures or by rIL2, like anergic cells. Second, and more importantly, the reactivity of the high reactive CD4+ T cells to TSHR-pulsed APCs was DQA*0501-restricted. Third, the CD8+ clones had an -T cell receptor (TCR), were CD3 positive, and CD28 negative. Last, mixing the highly reactive CD4+ cells and CD8+ cells resulted in a cell population containing both markers. When the last observation was examined functionally, it was observed that mixing the CD8+ cells with the high reactive CD4+ cells significantly attenuated the TSHR-pulsed APC response of the high reactive CD4+ cells. The suppression required cell-cell contact, was associated with the formation of a population of CD8+/CD4+ T cells by FACS analysis, was blocked by a T cell receptor antibody, but persisted for 24 to 48 hours after the CD8+ cells were removed from the CD4+ cells by magnetic beads coated with anti-CD8+ antibodies.

These results indicate that self-tolerance is maintained, in normal individuals, by CD8+ suppression of CD4+ cells highly reactive with TSHR-containing APCs. This involved binding of the CD8+ to the CD4+ cells. The bound complexes of CD8+/CD4+ T cells were, however, in equilibrium with free CD8+ and CD4+ T cells because they could be separately cloned. The anergic cell population is the CD4+ cells just released from the CD8+ cells at the time of expansion. In the case of a thyrocyte insult by virus infection, bacterial infection, or tissue injury, the increase in MHC class I induced by the double-stranded DNA or RNA in the cytoplasm might cause homing of the CD8+ suppressor cells to the thyrocytes with abnormal elevations of MHC class I. Subsequently, the CD4+ cells which are activated by release from the

CD8+ suppressor cells could interact with the TSHR on the thyrocyte-APC in the context of increased or aberrant class II.

The potential importance of this model is that it resolves multiple conflicting observations. It establishes a rational for the importance of MHC class I in the development of autoimmune disease. It is consistent with the importance of MHC class II for the development of stimulating TSHR antibodies. It is also consistent with the finding that Graves disease can develop in an animal with a normal immune system, if the target tissue becomes an APC. It is further consistent with the importance of MHC class I in the induction of an APC configuration by viruses, infections, or tissue injury. It may be additionally consistent with data showing that the ratio of suppressor-cytotoxic CD8+ cells to CD4+ cells was higher in intrathyroidal immune cell populations than in peripheral blood monocytes. This is discussed in a publication by Davies and co-workers (Davies, T. F., Martin, A., Concepcion ES, Graves, P., Cohen, L., and Ben-Nun, A. (1991) N. Engl. J. Med. 325:238-244; copy enclosed herewith).

In addition, two reports have positively associated HLA-DQA1*0501 with Graves disease. (Yanagawa, T., Mangklabruks, A., Chang, Y. B., Okamoto, Y., Fisfalen, M. E., Curran, P. G., and DeGroot, L. J. (1993) J. Clin. Endocrinol. Metab. 76:1569-1574; and Barlow, A. B. T., Wheatcroft, N., Watson, P., and Weetman, A. P. (1996) Clin.Endocrinol. (Oxf.) 44:73-77; copies enclosed herewith). Thus, the autoimmune response is the end result of a loss of normal self protection and is the means to resist cell infections and injuries. Applicants respectfully submit that this response is a totally protective response.

The Examiner also asserted that claims encompassing an *in vivo* method falls within the realm of genetic immunization which at the effective filing date of the present application was still immature and highly unpredictable. Applicants respectfully disagree with the Examiner's

remarks concerning DNA vaccines. These *in vivo* results couple DNA with an antigen the experimenters felt would be important therapeutically. In most cases this was an assumption wherein the error could well have been the antigen itself not the immunization with double-stranded polynucleotide. Moreover, the incorrectness of the Examiner's assertion is illustrated in the publications on gene therapy cited in the Office Action. Gene therapy involves immunization with a plasmid or viral construct in most cases, i.e. double-stranded polynucleotide. Currently, gene therapy does not work because patients develop an immune response to the plasmid antigen. Most research on gene therapy today presumes they can engineer the viral DNA to eliminate this problem. The present patent application provides evidence showing this is not necessarily the case. Thus, the double-stranded DNA will always generate an immune response. The Examiner accepts the fact that the Applicants can do this to generate a pathologic response. This is exactly the case in gene therapy. Thus, the Examiner's assertion that it is unlikely that Applicants can generate an immune response is incorrect. The issue then becomes is there sufficient disclosure in Applicants invention to enable a protective immune response. Applicants submit that this is so. Applicants have practiced the invention using the thyroid tumor regression model and have shown that the autoimmune response is by definition protective not pathologic.

The thyroid tumor model illustrates the second aspect of why the Examiner's assertion is incorrect and the cited Chattergoon reference is irrelevant. It is well recognized that critical autoantigens are cryptic. Thus many protective responses do not occur because the antigen that is important to develop the protective response is cryptic and unknown. In the thyroid tumor model Applicants allow the tumor itself to select the array of autoantigens it will present to

immune cells. Applicants respectfully submit that this will allow cells to present the cryptic autoantigen generating an autoimmune response.

Applicants further submit that in the Graves model, the TSHR is a known autoantigen causing an immune response. The selection of the TSHR which is a known autoantigen is not random. Thus, using the same immunization procedure plus thyroid peroxidase (TPO), the Rapoport group generated autoantibodies to TPO that for the first time mimicked the properties of autoantibodies to TPO in autoimmune thyroiditis (Hashimoto's disease). (Jaume, J. C., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S. M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657; copy enclosed herewith). More importantly, it emphasizes the point that the use of a known autoantigen can generate an autoimmune response. In tumors, Applicants do not know the identity of the autoantigen. That is the basis for failures of most studies atempting to develop an autoimmune response against tumors, i.e. immune therapy. In the current model Applicants do not guess the identity of the important autoantigen. Applicants respectfully submit that the instant invention discloses that the cell itself could do this as a normal protective response. Thus, contrary to the Examiner's assertion, undue experimentation will not be required to develop an autoimmune response.

The Examiner further states that results in animal systems are not predictive of outcome in applications to other species or humans citing Ledley as an example. Applicants respectfully submit that the quote from Ledley is taken out of context. Certainly animal experiments are not 100% predictive of what will occur in humans but animal experiments are required before human experimentation is commenced. In fact they are considered a predictive requirement by the FDA, i.e. a positive result in animal experiments is required before such human experiments can be carried out. Ledley discloses virus targeting and suggests that animal experiments are not

predictive because the animals do not have the viral attachment site. In no way does Ledley otherwise negate the importance of animal models. Thus, animal experiments if positive are often predictive of success in humans.

In the present invention, Applicants use a ds polynucleotide and have shown it works in many mouse and human cells, both primary cells or cells in continuous culture. See pages 50-52 of the specification. This argues strongly that this will be useful in human as well as mouse systems. To argue against that is nonscientific. The essence of the scientific process is to provide step-wise experimentation. If an experiment works in a mouse cell, it must be tested in a human cell to extrapolate results. This was done. If an experiment was done *in vitro*, it must also be tested *in vivo* to make the extrapolation. The Examiner appears to suggest that phase III clinical trials in humans must be successfully conducted in humans before an application for a patent can be filed in the United States Patent and Trademark Office. Applicants respectfully suggest that this an unreasonable burden and is not the law. Applicants respectfully suggest that the Examiner has placed an unreasonable burden on Applicants that they must prove that the invention is applicable in any and all subjects, when the Applicants have shown it is a likely and reasonable response in which appropriate predictive experiments have been performed. Applicants have disclosed that the invention is positive in multiple cells, human as well as mouse, that the invention works in vivo to produce an autoimmune disease, and the autoimmune response is a host protective mechanism.

The Examiner incorrectly asserts that the instantly claimed invention is a DNA vaccine. The principle underlying the instant invention is different. The Examiner asserts that specification fails to provide any guidance regarding delivery to a specific target cell. The Applicants respectfully submit that the Examiner is incorrect. It appears that the Examiner

believes that the pathologic immune response leading to disease is actually a protective response of the organism gone awry.

In the instantly claimed invention, Applicants do not target a cell *in vivo*, but rather Applicants treat the cell with the double-stranded DNA in vitro (tissue culture), establish that the cell becomes an APC in vitro (tissue culture), destroy the cell with mitomycin, then inject the modified cell *in vivo* intraperitoneally in the Graves or tumor model. Thus, Applicants respectfully submit that the invention is not about scientists or physicians targeting, but rather the individual's immune cells are allowed to target to the injected cells. Applicants' invention permits the host defense mechanism to do what it does normally to protect the organism. The increased class I removes the suppressive CD8 population, the CD4 population attacks, and an autoimmune response to an autoantigen in the injected cells is initiated. In the case of a vaccine, the analogous point holds true, as the vaccine is injected into muscle cells, these become APCs, they initiate the bystander immune response, and immune cell activation is unleashed to create antibodies to protect the organism. Applicants used ovalbumin in the Iishi reference, but this is readily done with tetanus toxin; the ovalbumin is a convenient analog. This is demonstrated in our earlier patents where antitetanus and antiovalbumin responses were measured. See, Singer, D. S., Kohn, L. D, Mozes, E., Saji, M., Weissman, J., Napolitano, G., Ledley, F. D.:Methods for assessing the ability of a candidate drug to suppress MHC class I expression. U.S. Patent 5,556.754, Feb. 17, 1996; and Singer, D. S., Kohn, L. D, Mozes, E., Saji, M., Weissman, J., Napolitano, G., Ledley, F. D.:Methods of treating autoimmune disease and transplant rejection. U.S. Patent 5,871,950 Feb. 16, 1999. Applicants submit that the ovalbumin response is widely accepted as predictive of a tetanus response.

Thus, Applicants respectfully submit that the Examiner incorrectly asserts that since the prior art does not provide such guidance nor the instant specification supply such teaching, it would therefore have required undue experimentation without a predictable expectation of success for one skilled in the art to make or use the claimed invention.

The Examiner asserted that the specification fails to provide teachings to claims drawn to an *ex vivo* method regarding the following issues:

> "What is the minimum proportion of cells or tumor cells transfected with a sequence non-specific ds-polynucleotide required to induce an effective protective or therapeutic immune response, or a desired autoimmune response in a host to generate various models for specific autoimmune conditions or diseases?", "To which host tissues do transfected cells home in and how long do they need to stay in the system of the host to induce desired immune responses?", "Which route of delivery of these transfected cells is effective to obtain the desired immune responses?", "How stable is the state of activated antigen presenting for cells transfected with a nonsequence specific ds-polynucleotide?"

Applicants respectfully submit that the Examiner incorrectly concludes that it would require undue experimentation for a skilled artisan to make and use the broadly claimed invention.

Concerning the Examiner's question of what is the minimum proportion of cells, Applicants respectfully submit that on page 94 of the specification it discloses that $1x10^7$ cells was effective. As disclosed in the specification, this procedure is a direct adaptation of Shimojo et al, PNAS, 93:11074-11079 (1996) (copy enclosed herewith). The Examiner should also note that in Shimojo et al a minimum number of cells was tested ($1x10^5$ and $1x10^6$) and a $1x10^6$ minimum defined. The tumor protective model uses the same number of cells effectively.

Concerning the Examiner's question of which host tissues do transfected cells home, Applicants respectfully submit that the cells do not target host tissues, but rather they remain intraperitoneal and immune cells target to them. Concerning the Examiner's question as to how

long do they need to stay in the system of the host to induce desired immune responses, Applicants submit that on page 94 of the specification it discloses that 6 times every two weeks, i.e. 12 weeks provides an effective immune response. This is the same duration of time described for the Shimojo model and the tumor model.

Concerning the Examiner's question of how stable is the state of activated antigen, Applicants respectfully submit that this is an irrelevant question in mitomycin killed cells (see page 94 of the specification) and is irrelevant *in vivo*, since the procedure is effective. Applicants submit that the state of activated antigen is sufficiently stable to produce an effective immune response in vivo under the procedure described.

Applicants respectfully submit that the instantly claimed invention provides sufficient guidance demonstrating an effective therapeutic or protective immune response. Applicants show a clear immune response in multiple models, Graves and now tumor treatment. The Examiner appears to construe Graves as pathologic not protective. Applicants submit that this is not the case. The pathologic response starts as a protective response to eliminate damaged or viral infected cells. The disease is a pathologic overshoot of the initial protective response. The tumor model is a disease model that is protective. Applicants teach how to create an autoimmune disease killing the tumor or its cells akin to diabetes wherein the islet cells are destroyed. With normal islet cells this kills them and is a disease. With tumor cells the same result is protective. Applicants respectfully submit that the Examiner must recognize that protective and therapeutic response are relative.

The Examiner incorrectly asserts that the specification fails to provide guidance for the generation of any and all other autoimmune disease models. As pointed out, the Shimojo model has been adapted to produce autoimmune thyroiditis using TPO as an autoantigen. (See: Jaume,

J. C., Guo, J., Wang, Y., Rapoport, B., and McLachlan, S. M. (1999) J. Clin. Endocrinol. Metab.84:1651-1657; copy enclosed herewith). The ds DNA model is, in retrospect, similarly effectively replicated in Castoglia, S., Rodien, P., Many, M-C., Ludgate, M., and Vassart, G. (1998) J. Immunol. 160:1458-1465 (copy enclosed herewith) where they injected plasmid plus TSHR. Applicants submit that this model is sufficient to enable one of ordinary skill in the art how to make and use the instantly claimed invention.

The Examiner asserts that relevant information concerning the cotransfected antigen, promoter, vector, cell dosage used, the frequency and route of administering utilized to generate other autoimmune disease models are absent. Applicants respectfully submit that the Examiner's assertion is incorrect. Given the close analogy of the instant invention to the Shimojo model, TPO in thyroiditis and an unknown thyroid tumor antigen in the thyroid tumor model are provided as examples. Applicants respectfully submit that because some experimentation may be required that does not rise to the level of undue experimentation. In addition, given the fact that Applicants teach that the choice of the nucleotide sequence is not critical, the promoter and vector need not be specified - any ds polynucleotide will work in the instantly claimed invention. Cell dose ($1x10^7$) is provided and used in the multiple Shimojo based models upon which the dsDNA Graves model is based.

The Examiner also asserts that guidance for overcoming differences in anatomy, cell biology, genetics, and immunology between animals is not provided. Applicants respecfully submit that differences in anatomy are irrelevant since intraperitoneal or intramuscular injections are used; this is not gene targeting of specific organs. Differences in cell biology are also irrelevant since multiple cell types and mouse or human cells are shown to have the same ds

nucleotide response (see pages 50-52 of the specification). In addition, the supplemental paper by Molteni enclosed herewith shows the immunologic basis in normal humans.

In summary, Applicants submit that the Examiner's conclusion that the specification does not provide sufficient direction and guidance for one skilled in the art to make and use the instantly claimed invention appears to be premised on the following: (a) a misunderstanding of the present invention (this is not gene therapy); (b) a misunderstanding of the nature of an autoimmune disease (it is a protective immune response which overcomes self-tolerance and becomes a disease in selected individuals); and (c) a misunderstanding of the clear experimental analogy to the Shimojo model which has been replicated by others and is applicable to other autoimmune responses, not only Graves disease. Thus, Applicants respectfully submit that in view of the above mentioned amendments and remarks Claims 1, 2, 4-18, 21-26, 29-35, 42-46, 74 and 75 are fully enabled and are in condition for allowance.

The Examiner asserts that Claims 60, 62 and 76-80 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner also asserts that the specification is not enabled for the claimed invention because the specification fails to provide guidance and direction for a skilled artisan to obtain any therapeutic effects for any specific diseases using the method or using the vaccine of the present invention as claimed.

The Examiner further asserts that given the failure of the instant specification to provide the specifics for carrying out the claimed method and use of the claimed vaccine for any of the

numerous claimed diseases, it would have required undue experimentation without a predictable expectation of success for a skilled artisan to make and use the claimed invention.

The Examiner also asserts that due to the lace of direction and guidance provided by the specification, the unpredictability of the genetic immunization art, and the breadth of the claims, it would have required undue experimentation without a predictable degree of success for one skilled in the art to make and use the instant broadly claimed invention.

The Examiner asserts that with regard to Claim 77, the specification fails to teach specifically the parameters involved in the increasing or decreasing expression of an antigen in a cell a particular antigen for a specific disease to be treated in the claimed method.

Applicants respectfully submit that the specification teaches the parameters and show that a specific tumor antigen does not need to be defined but rather Applicants can take advantage of a host cell defined auto antigen in the tumor model. Applicants submit that they have shown that the double-stranded DNA effect is additive and independent of cytokines (see Fig. 5 of the specification). Applicants further show in the Molteni reference (copy enclosed herewith) the immune response in humans that overcomes self-tolerance does not appear to be cytokine dependent but rather class I and class II dependent (CD8 and CD4 cell, respectively). Applicants respectfully submit that because some experimentation may be required that does not rise to the level of undue experimentation.

Thus, Applicants respectfully submit that in view of the above mentioned amendments and remarks Claims 60, 62 and 76-80 are fully enabled and are in condition for allowance.

D. Indefiniteness Rejection

The Examiner has asserted that Claims 1-2, 4-18, 21-26, 29-35, 42-46, 62, 74-76 and 78-80 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner has stated that in Claim 1, the phrase "expression of a gene, or gene and gene product, or gene product" is unclear and it seems to be redundant and clarification is needed. The Examiner has also stated that in Claim 1, there is an improper Markush language recited as "peptide processing genes or gene products consisting of TAP-1, TAP-2, a proteosome subunit, Class II regulatory genes and gene products. The Examiner has further stated that the term in Claim 1 "a STATs activation" is unclear, it does not represent a gene or a gene product in the recited group of the costimulatory gene or gene products and thus clarification is needed.

While applicants disagree with the Examiner, in order to eliminate any confusion, applicants have amended Claim 1. Applicants respectfully submit that expression of a gene refers to altered (increased or decreased) gene expression which includes the change in gene product. Expression of a gene product refers to expression of a protein by post transcriptional modification, such as in phosphorylation of STAT, altered dimerization of NF kappa B. Thus, expression of both gene and gene product concerns both together. Applicants refer the Examiner to pages 47 to 61 in example 1 of the specification where gene and gene product expression is altered and is discussed extensively, and to pages 61 to 62 in Example 2 of the specification which specifically discusses gene activation.

The Examiner has stated that in Claim 2 and its dependant Claims 15-18 and 24 there is insufficient antecedent basis for the recited term "the molecule." Applicants have amended Claim 1 to recite "gene or gene product" rather than the term "molecule."

The Examiner has stated that the phrase "an exogenous or environmental stimulus" is unclear and indefinite. Applicants respectfully disagree with the Examiner as the specification is replete with examples of exogenous or environmental stimulus. For example originally filed claims 9, 10, and 11 recite "viral infection," "bacterium, virus or cell" and "oncogene transformation" as examples of exogenous or environmental stimulus.

The Examiner has stated that Claims 32 and 34 have insufficient antecedent basis for the term "activated APC." Applicants are confused by the Examiner's assertion since Claims 32 and 34 depend on Claim 28 which provides sufficient antecedent support in the term "activated antigen presenting cell (APC). Applicants are similarly confused by the Examiner's assertion that there is no recitation of activated APC in claims 75 and 74 from which claims 32 and 34 are dependent upon respectively, since Claim 74 provides sufficient antecedent support in the term "activated antigen presenting cell (APC).

The Examiner has stated that Claim 46 is confusing and unclear whether the claim is directed to a composition or to a method because method steps are recited in the claim. Applicants have amended Claim 46 to recite a method claim.

The Examiner has stated that it is unclear whether claim 76 is drawn to a vaccine or a method of vaccination because the claim recites method steps. Applicants have amended Claim 76 to recite a method claim.

The Examiner has stated that Claim 74 is incomplete because it lacks a step or steps connecting the recited step to increasing presentation of antigen recited in the preamble of the claim. Applicants have amended Claim 74 accordingly.

The Examiner has stated that the recitation "enhance other treatment methods" in Claims 62 and 78 is unclear. Applicants have amended Claims 62 and 78 to recite "enhance other treatment methods that enhance an immune response or antigen presentation."

The Examiner has stated that there is insufficient antecedent basis for the limitation "activation or maturation of dendritic cells or peripheral blood macrophages" in claim Claim 79 and that in claims 77 and 78 from which claim 79 is dependent on, there is no recitation of dendritic cells or peripheral blood macrophages, but only of diseased cells removed from a mammal. Applicants have canceled Claim 79 and added new Claim 83. Applicants respectfully submit that support for the recitation macrophages and dendritic cells is disclosed in pages 49-51 of the specification where it is taught that macrophages and dendritic cells were used either as primary or continuous culture lines. Additional support for this recitation is disclosed in the results of Example 1 on page 57 of the specification, where Applicants teach these cell types had the same changes as thyrocytes.

The Examiner has stated that there is insufficient antecedent basis for the limitation "somatic cells" and "CpG residues" in Claim 80 and in Claims 77 and 78 from which claim 80 is dependent on. Applicants have accordingly amended claim 80. Applicants submit that support for this amendment is presented on pages 59-60 of the specification which discloses distinguishing double-stranded DNA from CpG residue action and pages 21-23 of the specification which discuss CpGs. Applicants submit that the combination of these two methods working on different cell types by different mechanisms is a method of enhancement of the immune response.

E. Closing

Attached hereto is a marked-up version of the changes made to the Claims by current amendment. The attached pages are captioned **"Version with markings to show changes made."**

The Claims have been amended to clarify the claim language and to more particularly point out applicants invention. No new matter has been added. In view of the foregoing remarks and amendments, it is submitted that none of the rejections can be sustained and that all should be withdrawn.. Claims 1, 2, 4-18, 21-26, 29-35, 42-46, 60, 62, 74-78 and 80, and new Claims 81-83 are in condition for allowance; reconsideration and allowance are respectfully requested.

If any matter requires attention prior to the allowance of the application, the Examiner is requested to contact the undersigned to resolve such matters.

Respectfully submitted,

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I hereby certify that this paper is being deposited this date with the U.S. Postal Service as first class mail addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Gerard P. Norton July 19, 2001

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Version with markings to show changes made.

1. (Amended twice) A method of increasing immune recognition of a mammalian cell by artificially introducing a sequence non-specific double-stranded polynucleotide greater than 25 nucleotides in length into the cell and thereby activating expression of a gene, or gene and gene product, [or gene product] that increases an immune recognition gene, or gene and gene product, or gene and gene product [including] comprising MHC class I and class II genes or gene and gene product[s], a peptide processing gene[s] or gene product[s] consisting of TAP-1, TAP-2, a proteosome subunit, Class II regulatory gene[s] and gene product[s] consisting of HLA-DM and invariant chain, costimulatory molecule[s] gene or gene products consisting of B7 costimulatory molecule, PKR, IFN-beta, MAP kinase, NF-κB, JAK, and [a] STATS [activation] wherein activation or postranslational modification of a gene product comprising MAP kinase, NF kappa B, JAK, and STATS, wherein [such] activation is further involved in antigen presentation, growth, and function of the cell, and which increases the ability of a cell to present antigen to an immune cell.

2. (Amended once) The method of claim 1 wherein the [molecule] gene or gene product is derived from the major histocompatibility complex (MHC).

46. (Amended twice) A [somatic mammalian cell with the enhanced ability to present] method of presenting antigen to the immune system of a mammal comprising;

(a) introducing [the] double-stranded polynucleotide into [the] a somatic mammalian cell with the enhanced ability to present antigen ex vivo, which improves the ability of the mammalian cell to present antigen; and

(b) measuring an increase in expression of an MHC molecule[s] or costimulatory molecule[s], or an MHC molecule[s] and a costimulatory molecule[s] involved in antigen presentation selected from the group consisting of TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, CIITA, RFX5, B7 costimulatory molecule, PKR, IFN-beta, MAP Kinase, NF-κB, JAK, and [a] STAT.

60. (Amended twice) A method for treating a mammalian disease [cancer, or an infectious disease caused by a virus, bacteria, yeast, protozoa, a disease caused by environmental injury or an autoimmune disease] which is sensitive to immunotherapy which comprises:

(a) removing diseased cells from a mammal;

(b) introducing a sequence non-specific double-stranded polynucleotide greater than 25 nucleotides in length into the cells;

(c) treating the cells to prevent cell division but permits other metabolic activity; and

(e) immunizing the mammal with the an effective amount of the cells to prevent or alleviate the symptoms of the disease.

62. (Amended twice) The method of claim 60 wherein the method of treatment is used to enhance another treatment method[s] [that] which further enhances an immune response or an antigen presentation.

74. (Amended twice) A method for increasing presentation of antigen by a mammalian cell comprising:

(a) introducing a sequence non-specific double-stranded polynucleotide greater than 25 nucleotides in length into the mammalian cell *ex vivo*, which causes the cell to have an increased ability to present antigen; [and]

(b) increasing the mammalian cell's ability to present antigen and forming an activated antigen presenting cell (APC); and

(c) measuring an increase in expression of an MHC molecule[s] or a costimulatory molecule[s], or an MHC molecule[s] and a costimulatory molecule[s] involved in antigen presentation selected from the group consisting of TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, CIITA, RFX5, B7 costimulatory molecule, PKR, IFN-beta, MAP Kinase, NF-κB, JAK, and [a] STAT.

76. (Amended once) A method for treating cancer [atherosclerosis, an autoimmune disease, or an infectious disease caused by a virus, bacteria, yeast protoza,] with a vaccine, comprising a somatic mammalian cell with the enhanced ability to present antigen to the immune system comprising;

(a) introducing a sequence non-specific double-stranded polynucleotide greater than 25 nucleotides in length into the somatic mammalian cell ex vivo, which causes the cell to have an increased ability to present antigen;

(b) measuring an increase in expression of MHC molecules or costimulatory molecules involved in antigen presentation selected from the group consisting of TAP-1, TAP-2, a proteosome subunit, HLA-DM, invariant chain, CIITA, RFX5, B7 costimulatory molecule, PKR, IFN-beta, MAP Kinase, NF-κB, JAK, and a STAT; and

(c) preparing the mammalian cell for immunization.

77. (Amended once) A method for treating cancer [,artherosclerosis, an infectious disease caused by a virus, bacteria, yeast, protozoa, a disease caused by environmental injury or an autoimmune disease] which is sensitive to immunotherapy which comprises:

(a) removing a diseased cell[s] from a mammal;

(b) increasing or decreasing the expression of antigen by the cell; and

(d) immunizing the mammal with an effective amount of the cell to prevent or alleviate the symptoms of the disease.

78. (Amended once) The method of Claim 77 wherein the method of treatment is used to enhance another treatment method[s] that enhances an immune response or an antigen presentation.

80. (Amended once) The method of claim [78] 76 wherein the treatment involves somatic cells and is coordinate with a treatment[s] with CpG residues used to enhance immune cell responsiveness.